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16. ABSTRACT

Halogenated aliphatic and aromatic hydrocarbons are the most important organic contaminants of shallow water-table aquifers. Such aquifers have recently been shown to harbor an indigenous microflora. Organisms in authentic and uncontaminated samples of unconsolidated subsurface material degraded styrene and toluene. Samples from one site also degraded chlorobenzene. Samples from three sites could not degrade tri- or tetrachloroethylene, 1,1,1- or 1,1,2-trichloroethane, 1,1- or 1,2-dichloroethane or chloroform at rates greater than 2% per week.

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INTRODUCTION

Ground-water pollution by organo-chlorine compounds can be resolved into two distinct problems. The first is a very extensive contamination of ground water in the industrialized world by a series of halogenated aliphatic hydrocarbons-primarily carbon tetrachloride, chloroform, trichloroethylene, tetrachloroethylene, and 1,1,1-trichloroethane. The frequency of detection of these compounds in samples from ground-water pumping stations in the Netherlands ranged from 17% of samples for 1,1,1-trichloroethane to 67% of samples for trichloroethylene (Zoeteman et al., 1981). Similarly, the U.S. EPA's Community Water Supply Survey showed that 45% of the public water supplies serving over 10,000 people from ground-water or combined ground-water and surface-water sources contain detectable concentrations of volatile organic compounds (U.S. EPA, 1982).

Although this pollution is extensive, it is not intensive, because concentrations of organo-chlorine contaminants are typically low. When 670 wells were sampled in New Jersey, U.S.A., the frequency of detection of the five important organo-chlorine pollutants ranged from 14% of samples for chloroform to 26% of samples for trichloroethylene. However, only 3% of the wells had concentrations of one or more chemical in excess of 100 µg/liter (Tucker, 1981). Typical concentrations are probably too low to perturb the ecology of the subsurface environment.

The second problem in ground-water pollution by organo-chlorine compounds is a localized, but very intense, pollution from spills and waste-disposal sites. In these cases, the organo-chlorine compounds or other pollutants occurring

with them are usually sufficiently concentrated to perturb any naturally occurring microbial communities, leading either to adaptation to the wastes or an inhibition of the community.

This study addresses the first problem area. It assesses the potential for biodegradation of organo-chlorine compounds at concentrations that are unlikely to perturb native biological communities occurring in otherwise pristine subsurface environments. It would be inappropriate to extrapolate the results of this study to ground-water pollution problems of the second type.

Oxygenated ground water is more prevalent than was thought to be the case in the past (Winograd and Robertson, 1982). Our results depict the biological fate of organo-chlorine compounds in shallow, aerobic, water-table aquifers, and should not be extended to anaerobic subsurface environments.

OCCURRENCE OF MICROBES IN THE SUBSURFACE

According to the conventional wisdom of soil microbiology, the population density of microbes in soil declines rapidly with increasing depth, particularly below the plow layer or the fertile top soil. This concept was derived from work done early in this century which used plate counts on conventional culture media to enumerate the microbes (See Waksman, 1916). An extension of this reasoning lead to the commonly held belief (in the U.S.A.) that aquifers were essentially devoid of life.

Recently a technique has been adapted to allow direct microscopic enumeration of microbes in subsurface material (Ghiorse and Balkwill, 1983). Samples are stained with the dye acridine orange. If it is associated with non-living material, the dye fluoresces red when excited by blue light. When it is associated with the nuclear material of organisms, the dye fluoresces green, allowing the organisms to be easily distinguished from non-living material of the same size. Material was recovered from the subsurface using procedures that prevented contamination by surface microorganisms (Wilson et al., 1983a). Direct microscopic examination revealed surprisingly high densities of organisms in deeper regions of the unsaturated zone and in regions just below the water table (Table 1) at every site examined.

In material from below the root zone, there was no appreciable decline in the population density of organisms

with depth. There was surprisingly close agreement between numbers at the same site at different times of the year, and between replicate bore holes at the same site.

The densities of organisms in the subsurface are comparable to those found in eutrophic surface waters. For purposes of comparison, 1 g of dry material as reported in Table 1 usually occupied a volume of 1.1 to 1.6 cm^3 of subsurface space which originally contained 0.15 to 0.3 ml of pore water. Eutrophic Lake Mendota in Wisconsin, U.S.A. contained 0.3 to 3.0 x 10^6 cells ml^{-1} (Pedros-Alio and Brock, 1982). Counts in the Essex estuary range from 2 to 8 x 10^6 cells ml^{-1} (Wright, 1978). The densities found by direct examination of subsurface material are also comparable to densities of planktonic ground-water microbes sampled from wells or piezometers. Samples of shallow ground-water from a subalpine forest in Canada contained 0.2 to 2.8 x 10^6 cells ml^{-1} (Ladd et al., 1982). Samples of groundwater from shallow sandy and gravelly material in the valley of the river Fulda in the Federal Republic of Germany contained 2.0 to 3.7 x 10^6 cells ml^{-1} (Marxsen, 1981). Samples of anoxic ground water from a deep aquifer in St. Louis Park, Minnesota, U.S.A. contained 10^6 to 10^7 cells ml^{-1} (Ehrlich et al., 1983).

These dense populations of subsurface microbes require some dependable source of metabolizable organic carbon; the most likely source is dissolved organic carbon in recharge water. The median concentration of dissolved organic carbon in ground waters of the U.S.A. is approximately 0.7 mg l^{-1} (Leenheer et al., 1974). The range is 15 to < 0.1 mg l^{-1}. Much of the dissolved organic carbon in ground water is not readily degradable. For example, ground water under Marmot Basin near Calgary, Canada with an average of 5.9 mg l^{-1} of dissolved organic carbon contained concentrations of carbohydrates, amino-acids, phenolic compounds, hydrocarbons, and tannins and lignins that were very low: 0.021,from above the water table at the Lula site. In the material from Lula, the rate of disappearance was significantly greater than in the autoclaved material ($P = 0.95$), indicating a biological process. The somewhat slower rates of disappearance in the material from Pickett were greater than zero ($P = 0.95$),but were not statistically different from loss in the autoclaved material. As a result, a nonbiological process cannot be precluded. There was no detectable degradation in the material from Fort Polk or from below the water table at Lula.

In contrast,toluene was degraded in all the materials investigated. In material from Pickett and Fort Polk, rates of disappearance were similar to the rate of disappearance

of chlorobenzene in material from Pickett. In the material from Lula, the rate of disappearance of toluene was more than an order of magnitude greater. The rate of disappearance of styrene was also reasonably close to that of chlorobenzene in material from Pickett. The rate of disappearance of styrene in material from Fort Polk was greater than any possible disappearance of chlorobenzene, but there is no information on how much greater it might be.

The rates of disappearance of a number of halogenated aliphatic hydrocarbons are presented in Table 3. Disappearance of bromodichloromethane was detected in material from below the water table at the Lula site. The rate of disappearance was statistically different from that in autoclaved material ($P = 0.95$), indicating a biological process. Trichloroethylene and tetrachloroethylene disappeared in some of the samples, but the rates were low, and were not statistically different from loss in the autoclaved material. There was no statistically significant evidence for the loss of chloroform, 1,1-dichloroethane, 1,2-dichloroethane, 1,1,1-trichloroethane, and 1,1,2-trichloroethane ($P = 0.95$). Experimentally detectable rates of degradation varied from 6% to 1% $week^{-1}$.

To determine whether the low rates of disappearance of trichloroethylene and tetrachloroethylene were due to very low rates of biotransformation, or to an abiotic transformation such as hydrolysis, or to loss of the compounds from the microcosms, three autoclaved microcosms and four non-autoclaved microcosms that were constructed with material from below the water table at Pickett, Oklahoma were analyzed after 62 weeks of incubation. The microcosms were originally constructed for the experiments described in Wilson et al., (1983 b). They were not needed for that experiment, therefore they were saved for analysis at a much later date. Results are presented in Table 4.

There was a consistent loss of the chlorinated aliphatic hydrocarbons in both autoclaved and non-autoclaved material. Expressed as a first-order rate, losses ranged from 0.6 to 6% $week^{-1}$ for the five compounds. Loss rates for trichloroethylene and tetrachloroethylene were 1.0 and 1.5% $week^{-1}$, which accounted nicely for the observed disappearance of these compounds seen in earlier experiments (Table 3).

A comparison of the loss of the chlorinated aliphatic hydrocarbons in autoclaved or non-autoclaved subsurface material reveals that the rate of any potential biotransformation of these compounds in the material from the Pickett site must be very low, less than 0.3% $week^{-1}$ in all cases (Ta-

ble 4).

Using the purge and trap method for concentrating volatile compounds, followed by gas chromatography (U.S. EPA, 1979), the pore water of the microcosms was examined for potential products of a biologically mediated reductive dehalogenation, including vinyl chloride, 1,1-dichlorethylene, and 1,2-dichloroethylene. None were found at concentrations equal to 1% or less of the potential parent compound. Acid extractable and neutral extractable compounds were also extracted into methylene chloride, concentrated, and analyzed by gas chromatography-mass spectrometry. Again, no transformation products were found. Unfortunately, the sensitivity to chloroacetic acids, which are possible oxidation products of the alkenes, was too low to preclude them as a possible abiotic transformation product.

The rates of disappearance of trichloroethylene and tetrachloroethylene were close to rates of abiotic degradation reported by Dilling et al.(1975) of 1.6 and 2.0% $week^{-1}$ respectively. However, the rates of disappearance of 1,1,1-trichloroethane, 1,1-dichloroethane, and chloroform were also of the same magnitude. It is also possible that all five compounds were simply lost from the microcosm, perhaps through diffusion through the Teflon liners of the screw caps that sealed the microcosms.

COMPARISONS TO OTHER GROUND WATER SYSTEMS

Schneider et al. (1981) followed the disappearance of tetrachloroethylene and 1,4-dichlorobenzene after infiltration of water from the River Glatt into its bank. Their results are consistant with this study. There was no significant loss of tetrachloroethylene, even in water 115 meters from the river, while the concentration of 1,4-dichlorobenzene was significantly reduced after travel of only 5 meters, and was reduced an order of magnitude after travel of 14 meters. Piet et al. (1981) followed the transformation of organic compounds under aerobic conditions after recharge of Rhine river water into sand dunes. Concentrations of several chlorinated aromatic compounds were reduced after passage through the dunes, but the concentration of tetrachloroethylene was not. Sontheimer (1980) summarized results on removal of organo-chlorine compounds during bank filtration of Rhine river water. Removals of trichloroethylene, dichloroethane, and chloroform plus carbon tetrachloride were low,25%

or less, while removals of chlorobenzene and toluene were high, 95 and 80% respectively. Interestingly, the removal of tetrachloroethylene was also high, 65%.

These published reports, plus this study, present a consistent pattern. In aerobic groundwaters, simple chloro - aromatic compounds may be biodegraded. There is very little evidence of biological attack of chlorinated aliphatic hydro carbons, such as has been reported in strongly reducing ground waters by Bouwer et al., (1981) and Wood et al.,(1981).

ABSTRACT

Several shallow water-table aquifers have been shown to harbor indigenous microbes at densities of 10^7 to 10^5 organisms g^{-1} of aquifer material. If these organisms are active against organo-chlorine compounds,such densities are high enough to carry out appreciable degradation of organic pollutants. The capacity of the indigenous microbes to degrade organic pollutants was examined by incubating samples of sub - surface material with dilute solutions of a number of the organic compounds that frequently pollute ground water.

Samples were recovered from positions immediately above and below the water table at three sites in the USA. The subsurface material was amended with a suite of organic compounds at concentrations of approximately 1 mg of each com - pound l^{-1} pore water. The samples were collected and manipulated in a manner that prevented contamination of the mate - rial by surface microorganisms.All samples were aerobic when collected.

There was no detectable degradation of 1,1-dichloroethane, 1,2-dichloroethane, 1,1,1-trichloroethane, 1,1,2-trichloroethane, or chloroform. Tri- and tetrachloroethylene disappeared slowly; however, they also disappeared in autoclaved material, indicating that the loss was an abiotic pro - cess. Bromodichloromethane, chlorobenzene, toluene, and styrene degraded in at least some of the samples. Degradation did not occur in autoclaved material, which indicates that degradation of these compounds in subsurface material was a biological process carried out by microbes that were indi - genous to the subsurface environment.

TABLE 1

Population density of organisms in the subsurface environment

Site	Depth to water table	Subsoil	Just above water table	Just below water table
	meters	..... millions g^{-1} dry material		
Lula, OK[c]	3.6			
February 81		6.8 (4.9)[a]	3.4 (2.6)	6.8 (4.3)
June 81		9.8 (1.3)	3.7 (1.0)	3.4 (1.1)
Fort Polk, LA[d]				
Borehole 6B	6.0	3.4 (1.7)	1.3 (1.0)	3.0 (2.8)
Borehole 7	5.0	7.0 (7.8)	1.3 (1.7)	9.8 (0.7)
Pickett, OK[e]	5.0	ND[b]	ND	5.2 (3.1)
Conroe, TX[f]	6.0	0.5 (0.6)	0.3 (0.3)	0.6 (0.5)
Long Island, NY[f]	6.0	ND	ND	36 (31)
	3.0	170 (40)	ND	ND

a Arithmetic mean (standard deviation)
b ND = no data
c Wilson et al., 1983
d Ghiorse and Balkwill, 1983
e Ghiorse and Balkwill, 1982
f W.C. Ghiorse, personal communication

TABLE 2

Rate of disappearance of selected aromatic hydrocarbons in uncontaminated samples of the subsurface environment

Compound	Lula, OK		Pickett, OK		Fort Polk, LA	
	Above[a]	Below[a]	Above	Below	Above	Below
	... % initial concentration per week ...					
Chlorobenzene	5.4 ± 3.3	<0.9	0.9 ± 0.8	1.9 ± 1.3	<1.0	<1.9
(autoclaved)	<3.0[c]	<1.1	<1.5	<2.4		
Toluene	>91[d]	>97[d]	0.9 ± 0.8	2.4 ± 1.0	2.5 ± 0.9	3.3 ± 3.2
(autoclaved)	<2.4	<2.4	<1.4	<1.4	<1.2	<12[e]
Styrene	no data		2.3 ± 0.7	4.3 ± 3.6	3.8 ± 1.1	12.0 ± 5.6
(autoclaved)			<0.8	<2.5	<1.9	<12[e]

a Samples taken immediately above or below the water table.
b Slope of linear regression ± 95% confidence interval on slope.
c Slope of the linear regression was less than the detection limit. Detection limit calculated as the standard error of the slope multiplied by the appropriate value of student's t for a one-tailed test at the 95% confidence level.
d Concentrations were below the analytical detection limit by the first sampling period.
e This material is not intrinsically more variable.The data from the last sampling date was lost.

TABLE 3

Rate of disappearance of selected halogenated aliphatic hydrocarbons in uncontaminated samples of the subsurface environment

Compound	Lula, OK		Pickett, OK		Fort Polk, LA	
	Above[a]	Below[a]	Above	Below	Above	Below
	..... % initial concentration per week					
Bromodichloromethane	<2.7[b]	2.4 ± 0.8[c]				
autoclaved	<3.2	<2.3				
Chloroform			<1.0	<2.0	<1.1	<2.8
1,1-Dichloroethane			<1.2	<1.9	<1.2	<2.6
1,2-Dichloroethane	<3.4	<3.5				
1,1,1-Trichloroethane			<1.1	<1.8	<1.2	<3.2
1,1,2-Trichloroethane	<4,8	<6,2				
Trichloroethylene	<2.9	<3.3	1.3 ± 1.1	<1.3	<1.2	<2.3
autoclaved			<1.5			
Tetrachloroethylene	<2.9	<4.4	1.1 ± 0.8	1.1 ± 1.0	0.9 ± 0.8	<1.8
autoclaved			<1.2	<2.0	<1.8	

a Samples taken immediately above or below the water table.
b Slope of the linear regression was less than the detection limit.
c Slope of a linear regression ± 95% confidence interval on the slope.

Lula data from Wilson et al., 1983a
Pickett and Fort Polk data from Wilson et al., 1983b.

TABLE 4

Disappearance of selected chlorinated aliphatic hydrocarbons in material from immediately below the water table at Pickett, OK

Compound	Recovery of Spike %	Observed concentration (% added concentration)			Increase in rate of disappearance in material that was not autoclaved (% per year)	Increase in rate of disappearance required at the probability level (% per year)
		Zero Time	After 62 weeks Autoclaved	After 62 weeks Not autoclaved		
1,1,1-Trichloroethane	66.6	63.9 ± 29.2[a]	49.8 ± 13.7[a]	40.3 ± 11.4[a]	7.9	10.5
Tetrachloroethylene	65.7	59.6 ± 25.2	30.5 ± 17.9	24.2 ± 17.8	6.3	18.0
Trichloroethylene	73.2	65.5 ± 26.0	26.6 ± 8.1	25.3 ± 13.3	1.3	12.5
1,1-Dichloroethane	No data	80.3 ± 34.3	31.4 ± 14.8	29.5 ± 3.1	1.9	4.6
Chloroform	108.8	76.1 ± 18.8	51.6 ± 10.4	52.8 ± 7.0	- 1.2	7.9

a Arithmetic mean ± 95% confidence interval on the mean.

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